ASSOCIATION OF GHRELIN GENE POLYMORPHISMS AND EXPRESSION LEVELS WITH SOME BIOCHEMICAL TRAITS IN BROILER CHICKENS

A. A. Joody I.l A. Abdul-Hassan* D. H. Al-hassani**

***Institute for Genetic Engineering and Biotechnology, University of Baghdad**

**** Department of Animal Production, College of Agriculture, University of Baghdad**

ABSTRACT

This study was conducted to investigate the association of Ghrelin gene polymorphisms and expression levels with some biochemical traits of broiler chickens. Two hundred broiler chicks Ross308, one-day old were wing-tagged and reared under optimal conditions. Blood samples were collected individually from all birds on 21 and 35 days of age. Proventriculus was collected at 35 days of age from thirty birds of each of groups sorted according to growth rate for high, moderate and low to measure ghrelin gene expression by real-time RT-PCR. The serum traits of glucose, uric acid, cholesterol, triglyceride, high density lipoproteins (HDL), low density lipoproteins LDL, total protein, albumin, globulin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were not influenced by ghrelin gene polymorphisms. While significant ($P < 0.01$) differences were recorded among the three chicken groups with high, moderate and low ghrelin expression in serum glucose, uric acid, total protein, albumin, cholesterol, triglyceride, HDL, AST, ALT and ALP concentrations at 21 and 35 days of age which increased with increasing ghrelin gene expression. The PCR-RFLP technique revealed no significant differences between GG and LL genotypes of ghrelin gene in all biochemical traits at 21 and 35 days of age, whereas ghrelin gene expression had a significant effect on serum glucose, uric acid, total protein, albumin, cholesterol, triglyceride, HDL, AST,ALT and ALP.

Keywords: slucose, uric acid, cholestcol, blood serum, proteins.

مجلة العلوم الزراعية العراقية –2018 :49(5):870-878 جودي وآخرون

علاقة طرز ومستوى جين الكرلين مع بعض الصفات الكيموحيويه لفروج اللحم

احمد عبودي جودي اسماعيل عبد الرضا عبد الحسن ضياء حسن الحسني**

مدرس أستاذ أستاذ

*معهد الهندسة الوراثيه والتقنيات الاحيائيه، جامعة بغداد

**قسم الانتاج الحيواني، كلية الزراعه، جامعة بغداد

المستخلص

انجزت الدراسه لغرض البحث عن العلاقه بين طرز ومستوى تعبير جين الكرلين والصفات الكيموحيويه لفروج اللحم .أستخدم 200 فروج لحم سلالة Ross 308بعمر 1 يوم، رقمت بالاجنحه وربيت في ظروف مثاليه .جمع الدم فرديا من جميع الطيور بأعمار 21 و 35 يوم .أخذت عينات المعدة الغديه من 30 طير من كل مجموعه من المجاميع المصنفه على أساس السريعه، المتوسطة والبطيئة النمو وذلك لقياس تعبير جين الكرلين بتقنية RT-PCR. أظهرت النتائج عدم تأثير طرزجين الكرلين على الكلوكوز، حامض البوليك، الكوليسترول، الدهون الثلاثيه، البروتينات الدهنيه عالية الكثافه HDL و واطئة الكثافه LDL، البروتينات الكليه، الالبومين، الكلوبيولين، انزيمات AST,ALT,ALD في سيرم الدم .في حين سجلت فروقات معنويه بين مجاميع تعبير جين الكرلين الثلاث في الكلوكوز، حامض البوليك، البروتينات الكليه، الالبومين، الكوليسترول، الدهون الثلاثيه، HDL,AST, ALT, ALPالسيرم الدم في عمري 21 و 35 يوم فقد ارتفعت مع زيادة التعبير الجيني. أظهرت تقنية تعدد اطوال قطع التقييد RFLP عدم وجود فروقات معنويه بين التراكيب الوراثيه GG و LL في جميع الصفات الكيموحيويه عند عمري 21 و 35 يوم بينما أظهر التعبير الجيني تأثيرا معنويا في الكلوكوز، حامض البوليك، البروتينات الكليه، الالبومين، الكوليسترول، الدهون الثلاثيه، HDL, AST, ALT, ALP.

كلمات مفتاحية: كلوكوز، حامض البوليك، كوليسترول، سيرم الدم، البروتينات.

***Received:27/2/2018, Accepted:22/5/2018**

INTRODUCTION

Ghrelin has passed seventeen years since its discovery in rats. At the present time, thousands studies were conducted in mammal and non-mammal animals to identify properties of ghrelin in most animal species. Broiler chickens are considered as a favorable animal for eperiments (2 ,13) defined ghrelin as the first endogenous ligand of the growth hormone Secretagogue receptor (GHS-R) that stimulates GH release. Ghrelin consists of 26 amino acids long (in most avian species) and sharing 54 % amino acid sequence identity with human and rat ghrelin which generally consists of 28 amino acids in mature ghrelin peptide (15). Since 1999 when the important role of ghrelin in promoting GH secretion was discovered, studies and research works trying to discover the existence and physiological functions of ghrelin in different organisms such as human, fish, mouse, rat and avian species were increased dramatically. In avian, gastrointestinal tract produces ghrelin hormone from enteroendocrine cells, which is distributed in most parts of gastrointestinal tract, especially in proventriculus, so these cells play essential role in control several processes related to food intake and food digestion, in consequence of effect on bird metabolism status through sensation several compounds like lipids, proteins, carbohydrates amino acids and others (6 , 14 , 31). Because signals are transferred by several sensors found on surface of these cells, ghrelin can be categorized as brain-gut hormone (8 , 15). The first signal that has inhibitory effect or orexigenic effect is produced by activation the axon terminals of neuropeptide Y (NPY), Gamma Aminobutyric acid (GABA) and Agouti-related peptide (AGRP) by activation GHS-R distributed on terminals of axon (7 , 24 , 30). NPY is considered the major neurotransmitter with orexigenic effect on food intake in avian species, NPY inhibits the activity of Proopiomelanococortin (POMC) and Cocaine-and amphetamine-regulated transcript (CART) through inhibitory action of GABA in the postsynaptic of Y1 and Y5 receptors that blocks nerve impulses (12 , 28), while releasing AGPR neurons leads to blocks of melanocortin 4 receptor (MC4R) which is defined as specific receptor for α-melanocyte stimulating hormone (α-MSH). Block MC4R leads to stimulating food intake in avian species, in other words, AGPR decreased binding α-MSH with MC4R during fasting or food restriction, therefore AGPR increased versus decreased in POMC (19 ,19, 29). There was a shortage in studies concerning ghrelin gene polymorphisms and expression in modern commercial strains and its association with some biochemical traits, therefore this study aimed to investigate the relationship between ghrelin gene expression and each growth rate level with some biochemical traits in broiler chickens type Ross 308.

MATERIALS AND METHODS

This study was conducted at AL-Aammeri hatchery and poultry farm in Mahaweli Area / City of Babel during a period from 3 April 2015 to 1 Jun 2015. Two hundred broiler chicks type Ross308 , one-day old, 41-44 grams average live body, were tagged individually by aluminum wing tags and reared in floor pen (2.5m×2.5m). Chicks feed and water were provided in *ad Libitum* during the experiment. Diets were formulated according to National Research Council (23) to meet feed requirements of starter and finisher rations for the birds (table 1). In order to boost birds' immunity, they were vaccinated against Newcastle and Gumboro diseases according to their age. A 4-5ml Blood samples were collected individually from wing vein of all birds on 21 and 35 days of age (1). Blood samples were divided into tubes coated with EDTA tubes without EDTA (AFCO –Jordan). The tubes without EDTA were centrifuged immediately with 8000 rpm for 15 minutes to separate serum which was decanted into sterilized glass vials, stoppered tightly and stored at -18 ºC until analysis. Serum glucose, total proteins, total proteins, triglycerides, cholesterol, HDL-cholesterol, LDL cholesterol, Uric acid, AST, ALT, ALP concentrations were determined by using commercial kits. Genomic DNA was extracted from 200 µl of frozen blood by using the gSYNC™ DNA Extraction Kit (Geneaid USA). The concentration of DNA samples and the purity was estimated by nanodrop. The PCR amplification for ghrelin gene was carried out using Accu Power PCR PreMix KIT (Bioneer, Korea). The sequence of

primers used to characteristic ghrelin gene (GenBank:JN578262.1) were, Forward; 5'AAGGACACGTGGAAACTGCCAGC3',Reverse;5'AAGCAGCCTGAGGTGACTGCAA3'. The amplification program were as follows: Initial denaturation at 94℃ for 3 minutes , followed by 35 cycles of denaturation at 94℃ fo 30 seconds, annealing at 60℃ for 45 seconds, and extension at 72℃ for 1 minute , with final extension at 72℃ for 5 minutes, then **hold** at 4℃ . The product was digested by *Hinf I* restriction enzyme. At 35 days of age, thirty chickens of each of high, moderate and low growth rates were selected and slaughtered to collect proventriculus, which was deep frozen in liquid nitrogen until measuring ghrelin gene expression. Total RNA was extracted from proventriculus tissue using Total RNA Mini Ki (Geneaid USA). Conversion of mRNA into double-stranded cDNA carried out by using AccuPower® RocketScript™ RT PreMix kit (Bioneer - Korea). Complementary DNA of ghrelin was amplified with the following primers: F: CCT TGG GAC AGA AAC TGC TC and R: CAC CAA TTT CAA AAG GAA CG with used chicken 18S ribosomal RNA F: CGCGTG CAT TTA TCA GAC CA, R: ACC CGT GGT CAC CAT GGT A (Gene Bank accession no AF173612) as an internal control in qPCR. AccuPower® GreenStarTM qPCR PreMix was used to mRNA expression quantified using the Exicycler™ 96 (Exicycler™ 96 Real-Time Quantitative Thermal Block, Bioneer Co.) apparatus according to following protocol: one cycle of Pre Denaturation 95 °C, 1-5 min, 40-45 cycles of Denaturation 95 °C, 5-20 sec, Annealing/Extension at 55-60 °C for 40-45 sec, Detection(Scan) then one cycle of Melting. After completion reaction, all data were obtained using Exicycler 96 Real-time Quantitative Thermal Block (Bioneer Co.). The CT for each sample was determined by Exicycler 96 detection software (version 1.2, Bioneer) and quantitative was calculating by 2-ΔΔCt method (20). Data were subjected to analysis of variance (26) and significant means were separated by Duncan's multiple range test (10).

Table 1. Ingredients and composition of the experimental diets

Ingredients	Starter ration (%)	Finisher ration (%)
Yellow corn	**37**	**45**
Soybean meal(48% protein)	**30**	**26**
Wheat	**26**	**20**
Protein concentrate[1]	**5**	**5**
Sunflower oil	**1.5**	**3.5**
Premix[**]	**0.1**	**0.1**
Salt	**0.3**	**0.3**
Dicalcium phosphate	**0.1**	**0.1**
Total	**100**	**100**
Calculated chemical analysis		
Metabolize energy (kcal/kg)	**2926**	**3097.8**
Crude protein (%)	**22.4**	**20.5**
Calcium (%)	**0.82**	**0.80**
Available phosphorus (%)	**0.61**	**0.58**
Methionine (%)	**0.61**	**0.58**
Lysine (%)	**1.74**	**1.63**

[1]protein concentrate contain; Crude Protein 40%, Crude fat 7.5 %, Crude fiber 3 %, Calcium 12%, phosphorus(av) 4.8 % methionine 3.7%, meth + cys. 4.0 %, Lysine 3.9 %, sodium 2.2 %, metabolizable Energy 2000 kcal/kg.

RESULTS AND DISCUSSION

Data exhibited that ghrelin gene polymorphisms did not affect glucose, uric acid, cholesterol, triglyceride, HDL, LDL, total protein, albumin, globulin, AST, ALT and ALP in blood serum of broiler chickens type Ross 308 at 21 and 35 days of age, mentioned in Table 2.

Table 2. Effect of ghrelin gene polymorphisms on some serum biochemical traits in Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Parameter	21 days		p value	35 days		p value
	GG	LL		GG	LL	
Glucose (mg/dl)	72.27 ± 1.09	72.31 ± 5.54	NS	168.42 ± 3.52	188.49 ± 18.54	NS
Uric acid(mg/dl)	5.03 ± 0.07	4.98 ± 0.38	NS	5.09 ± 0.10	5.49 ± 0.62	NS
Cholesterol(mg/dl)	99.25 ± 1.38	99.53 ± 8.25	NS	177.46 ± 3.67	195.09 ± 21.52	NS
Triglycerides mg/dl	136.73 ± 1.95	136.25 ± 12.74	NS	157.38 ± 3.26	173.92 ± 19.52	NS
HDL (mg/dl)	26.72 ± 0.43	27.46 ± 2.24	NS	50.94 ± 1.10	55.88 ± 6.50	NS
LDL(mg/dl)	45.18 ± 0.64	44.81 ± 3.51	NS	95.04 ± 1.94	104.42 ± 11.17	NS
T.Protein (g/dl)	3.71 ± 0.06	3.77 ± 0.42	NS	3.17 ± 0.07	3.43 ± 0.39	NS
Albumin(g/dl)	1.77 ± 0.03	1.78 ± 0.17	NS	1.41 ± 0.03	1.56 ± 0.17	NS
Globulin (g/dl)	1.94 ± 0.04	1.99 ± 0.28	NS	1.75 ± 0.04	1.87 ± 0.24	NS
AST (IU/L)	7.36 ± 0.12	7.57 ± 0.79	NS	7.36 ± 0.16	8.32 ± 0.92	NS
ALT (IU/L)	11.79 ± 0.25	13.20 ± 1.47	NS	9.28 ± 0.20	10.49 ± 1.20	NS
ALP (IU/L)	16.44 ± 0.23	16.52 ± 1.50	NS	16.35 ± 0.34	18.08 ± 2.16	NS

NS: No Significant difference, AST : Aspartate transaminase, ALT: Alanine transaminase, ALP : Alkaline phosphatase.

At 21 day s of age, serum glucose and uric acid concentrations of males and females with high ghrelin gene expression were significantly (P < 0.0.5) higher than those of low ghrelin gene expression, whereas at 35 days of age, the serum glucose and uric acid concentrations of male and female broilers with high ghrelin gene expression were significantly (p<0.01) higher than those of moderate and low ghrelin gene expression (Table 3). Our results were not in agreement with data mentioned by Lotfi *et al.*, (21), who revealed that injection 50 and 100 ng of exogenous ghrelin on 5 and 10 days of incubation had no significant effect on serum glucose of newly hatched chickens, since a significant effect was found in insulin concentration which increased significantly with the levels raise of ghrelin injection.

Table 3. Effect of three levels of ghrelin gene expression on serum glucose and uric acid in males and females of Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Age (Days)	Sex	Serum glucose (mg/dl)			p value
		High	Moderate	Low	
21	M	79.83 ± 1.55 ^{a}	72.98 ± 1.99 ^{ab}	68.31 ± 4.53 ^{b}	*
	F	78.01 ± 1.80 ^{a}	72.74 ± 1.99 ^{a}	64.03 ± 2.00 ^{b}	*
	Average	79.37 ± 1.23 ^{a}	72.85 ± 1.39 ^{b}	65.41 ± 1.98 ^{c}	**
35	M	209.40 ± 3.62 ^{a}	175.18 ± 3.76 ^{b}	147.47 ± 12.06 ^{c}	**
	F	192.55 ± 2.83 ^{a}	166.98 ± 2.37 ^{b}	130.14 ± 4.97 ^{c}	**
	Average	205.19 ± 3.16 ^{a}	170.87 ± 2.25 ^{b}	135.71 ± 5.24 ^{c}	**
		Serum uric acid (mg/dl)			
21	M	5.53 ± 0.11 ^{a}	5.03 ± 0.12 ^{ab}	4.70 ± 0.35 ^{b}	**
	F	5.42 ± 0.14 ^{a}	5.10 ± 0.12 ^{a}	4.52 ± 0.15 ^{b}	**
	Average	5.50 ± 0.09 ^{a}	5.07 ± 0.08 ^{b}	4.58 ± 0.15 ^{c}	**
35	M	6.26 ± 0.10 ^{a}	5.33 ± 0.12 ^{b}	4.41 ± 0.39 ^{c}	**
	F	5.93 ± 0.06 ^{a}	5.06 ± 0.09 ^{b}	3.90 ± 0.17 ^{c}	**
	Average	6.18 ± 0.08 ^{a}	5.19 ± 0.08 ^{b}	4.07 ± 0.17 ^{c}	**

Gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** mean a significant difference at p≤0.05 and p≤0.01 levels, respectively

These contradictory results can be explained by capability of insulin to interact directly with neurons such as ARC NPY that are considered important neurons to ghrelin action, in other word when serum glucose or insulin reachs to certain ratio with serum ghrelin concentration, the action of ghrelin in hypothalamus is inhibited (9 , 22 , 32). Table 4 demonstrates The serum total protein, albumin and globulin concentration values at 21 and 35 days of age for both sexes. At 21 days of age, the serum total protein concentrations of male broilers with high ghrelin gene expression were significantly (p<0.01) higher than those of low ghrelin gene expression (4.07 *versus* 3.51 g/dl respectively), whereas serum albumin concentrations of male broilers with high ghrelin gene expression were significantly (p<0.01) higher than those of moderate and low ghrelin gene expression (1.94 *versus* 1.78 and 1.69 g/dl respectively). Serum globulin concentrations for both broiler sexes with high

ghrelin gene expression were not significant different from those of low ghrelin gene expression. On the other hand, the serum total protein and albumin concentrations of 35 days old male broilers with high ghrelin gene expression were significantly ($p<0.01$) higher than those of moderate and low ghrelin gene expression. In general, serum total protein, albumin and globulin concentrations at 35 days of age increased along with the increase of ghrelin gene expression. At 21 days of age, the serum cholesterol and triglyceride concentrations of male broilers with high ghrelin gene expression were significantly ($p<0.01$) higher than those of low ghrelin gene expression, however were not significant with those with moderate ghrelin gene expression (table 5). Serum cholesterol concentration of females at 35 days of age with high ghrelin gene expression were significantly ($p<0.01$) higher than those of low ghrelin gene expression. In general, serum cholesterol and triglyceride concentration at 35 days of age are increase along with increasing ghrelin gene expression.

Table 4. Effect of different levels of ghrelin gene expression on serum total protein (g/dl), albumin and globulin in males and females of Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Age (Days)	Sex	Serum total protein (g/dl)			p value
		High	Moderate	Low	
21	M	4.07 ± 0.11 ^{a}	3.65 ± 0.13 ^{ab}	3.51 ± 0.27 ^{b}	**
	F	3.99 ±0.16 ^{a}	3.78 ± 0.01 ^{ab}	3.36 ± 0.13 ^{b}	**
	Average	4.05 ± 0.10 ^{a}	3.71 ± 0.08 ^{b}	3.41 ± 0.12 ^{c}	**
35	M	3.92 ± 0.07 ^{a}	3.29 ± 0.07 ^{b}	2.77 ± 0.22 ^{c}	**
	F	3.58 ± 0.09 ^{a}	3.16 ± 0.04 ^{b}	2.44 ± 0.11 ^{c}	**
	Average	3.83 ± 0.06 ^{a}	3.22 ± 0.04 ^{b}	2.55 ± 0.10 ^{c}	**
		Serum albumin(g/dl)			
21	M	1.94 ± 0.03 ^{a}	1.78 ± 0.04 ^{b}	1.69 ± 0.11 ^{b}	**
	F	1.90 ± 0.03 ^{a}	1.78 ± 0.04 ^{ab}	1.56 ± 0.05 ^{b}	**
	Average	1.93 ± 0.03 ^{a}	1.78 ± 0.03 ^{b}	1.60 ± 0.05 ^{c}	**
35	M	1.74 ± 0.3 ^{a}	1.47 ± 0.03 ^{b}	1.23 ± 0.10 ^{c}	**
	F	1.61 ± 0.02 ^{a}	1.41 ± 0.02 ^{b}	1.09 ± 0.04 ^{c}	**
	Average	1.71 ± 0.03 ^{a}	1.44 ± 0.17 ^{b}	1.13 ± 0.04 ^{c}	**
		Serum globulin(g/dl)			
21	M	2.13 ± 0.09 ^{a}	1.87 ± 0.10 ^{a}	1.83 ± 0.17 ^{a}	NS
	F	2.08 ±0.16 ^{a}	1.99 ± 0.07 ^{a}	1.79 ± 0.10 ^{a}	NS
	Average	2.12 ± 0.08 ^{a}	1.93 ± 0.06 ^{ab}	1.80 ± 0.08 ^{b}	**
35	M	2.18 ± 0.04 ^{a}	1.82 ± 0.04 ^{b}	1.54 ± 0.12 ^{c}	**
	F	1.97 ±0.07 ^{a}	1.75 ± 0.02 ^{b}	1.35 ±0.07 ^{c}	**
	Average	2.13 ± 0.40 ^{a}	1.79 ± 0.02 ^{b}	1.41 ± 0.06 ^{c}	**

Gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** mean a significant difference at p≤0.05 and p≤0.01 levels, respectively.

Table 5. Effect of different levels of ghrelin gene expression on serum cholesterol and triglycerides in males and females of Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Age (Days)	Sex	Serum cholesterol (mg/dl)			p value
		High	Moderate	Low	
21	M	108.60 ± 1.89 ^{a}	100.14 ±2.49 ^{ab}	94.26 ± 6.03 ^{b}	**
	F	107.41 ± 1.90 ^{a}	100.20 ± 2.17 ^{a}	88.38 ± 2.82 ^{b}	**
	Average	108.30 ± 1.48 ^{a}	100.17 ± 1.62 ^{b}	90.27 ± 2.71 ^{c}	**
35	M	219.44 ± 3.91 ^{a}	184.73 ± 3.95 ^{b}	155.15 ± 12.61 ^{c}	**
	F	202.40 ± 2.93 ^{a}	176.23 ± 2.58 ^{b}	137.57 ± 5.36 ^{c}	**
	Average	215.18 ± 3.37 ^{a}	180.25 ± 2.38 ^{b}	143.22 ± 5.54 ^{c}	**
		Serum triglycerides(mg/dl)			
21	M	150.50 ± 2.61 ^{a}	138.75 ± 3.46 ^{ab}	130.61 ± 8.16 ^{b}	**
	F	146.66 ± 3.57 ^{a}	137.74 ± 2.96 ^{a}	120.40 ± 4.09 ^{b}	**
	Average	149.54 ± 2.14 ^{a}	138.22 ± 2.23 ^{b}	123.68 ± 3.84 ^{c}	**
35	M	194.44 ± 3.23 ^{a}	164.50 ± 3.44 ^{b}	137.03 ± 11.38 ^{c}	**
	F	178.50 ± 2.90 ^{a}	157.06 ± 2.33 ^{b}	121.43 ± 4.71 ^{c}	**
	Average	190.45 ± 2.89 ^{a}	160.58 ± 2.10 ^{b}	126.45 ± 4.93 ^{c}	**

Gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** mean a significant difference at p≤0.05 and p≤0.01 levels, respectively.

At 21 days of age, the serum HDL concentrations of male and females with high ghrelin gene expression were significantly ($p<0.01$) higher than those of low ghrelin gene expression, whereas no significant differences was found with moderate ghrelin gene

expression (Table 6). Serum HDL and LDL levels at 35 days of age being increased along with the increasing ghrelin gene expression. The results are disagreed with results of Lotfi and Shahryar (27) who showed that ghrelin antagonist injected *in ovo* did not effect significantly in serum triglyceride concentration of broiler chickens. On the other hand a significant decrease was recorded in serum triglyceride levels of newly hatched chickens after 50 and 100 ng exogenous ghrelin *in ovo* injected at 5 or 10 days of incubation (21). Decrease of lipid parameters in low ghrelin gene expressed broiler chickens could be a result of increase glucagon releases in response to dropping glucose levels, hence the fatty acid synthesis is depressed by glucagon increase. Raise some lipid levels in chicken blood serum, especially triglycerides in high level ghrelin expressed chickens could be due to the role of these lipids in promoting transport of ghrelin across blood brain barrier (BBB), since the triglycerides is considered as one of the promoting factors for ghrelin transport in serum (4 , 5).

Table 6. Effect of different levels of ghrelin gene expression on serum High Density Lipoprotein (HDL- cholesterol) and LDL cholesterol concentrations in males and females of Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Age (Days)	Sex	Serum HDL(mg/dl)			p value
		High	Moderate	Low	
21	M	29.93 ± 0.72 ^{a}	26.55 ± 0.70 ^{ab}	25.83 ± 2.03 ^{b}	**
	F	28.33 ± 0.83 ^{a}	26.86 ± 0.80 ^{ab}	23.92 ±0.80 ^{b}	**
	Average	29.53 ± 0.60 ^{a}	26.62 ± 0.53 ^{b}	24.53 ± 0.84 ^{c}	**
35	M	63.46 ± 1.25 ^{a}	53.33 ± 1.26 ^{b}	44.58 ± 3.61 ^{c}	**
	F	58.17 ± 1.08 ^{a}	50.35 ± 0.83 ^{b}	38.95 ± 1.49 ^{c}	**
	Average	62.13 ±1.08 ^{a}	51.76 ± 0.77 ^{b}	40.75 ± 1.58 ^{c}	**
		Serum LDL(mg/dl)			
21	M	48.57 ±0.95 ^{a}	45.85 ±1.27 ^{ab}	42.31 ± 2.62 ^{b}	**
	F	49.74 ± 1.05 ^{a}	45.79 ±0.98 ^{a}	40.83 ± 1.37 ^{b}	*
	Average	48.87 ± 0.75 ^{a}	45.91 ± 0.78 ^{b}	41.00 ± 1.24 ^{c}	**
35	M	117.10 ± 2.08 ^{a}	98.50 ± 2.08 ^{b}	83.16 ± 6.76 ^{c}	**
	F	108.53 ± 1.64 ^{a}	94.47 ± 1.36 ^{b}	74.33 ± 3.00 ^{c}	**
	Average	114.96 ± 1.78 ^{a}	96.38 ± 1.25 ^{b}	77.17 ± 3.01 ^{c}	**

Gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** mean a significant difference at p≤0.05 and p≤0.01 levels, respectively.

Table 7. Effect of different levels of ghrelin gene expression on serum Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) concentrations in males and females of Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).

Age (Days)	Sex	Serum AST(lu/L)			p value
		High	Moderate	Low	
21	M	8.01 ± 0.22 ^{a}	7.74 ± 0.23 ^{ab}	7.12 ± 0.45 ^{b}	*
	F	7.96 ± 0.23 ^{a}	7.27 ± 0.24 ^{ab}	6.43 ± 0.22 ^{b}	**
	Average	8.00 ± 0.18 ^{a}	7.49 ± 0.17 ^{b}	6.65 ± 0.22 ^{c}	**
35	M	9.25 ± 0.17 ^{a}	7.73 ± 0.19 ^{b}	6.41 ± 0.39 ^{c}	**
	F	8.35 ± 0.11 ^{a}	7.32 ±0.12 ^{b}	5.57 ± 0.22 ^{c}	**
	Average	9.02 ± 0.15 ^{a}	7.52 ± 0.11 ^{b}	5.84 ± 0.24 ^{c}	**
		Serum ALT(lu/L)			
21	M	14.61 ± 0.25 ^{a}	12.35 ± 0.26 ^{b}	10.27 ± 0.89 ^{c}	**
	F	13.43 ± 0.15 ^{a}	11.70 ± 0.16 ^{b}	9.05 ± 0.37 ^{c}	**
	Average	14.32 ± 0.22 ^{a}	12.01 ± 0.16 ^{b}	9.44 ± 0.39 ^{c}	**
35	M	11.55 ± 0.21 ^{a}	9.79 ± 0.21 ^{b}	8.04 ± 0.62 ^{c}	**
	F	10.61 ± 0.14 ^{a}	9.25 ± 0.14 ^{b}	7.02 ± 0.29 ^{c}	**
	Average	11.32 ± 0.18 ^{a}	9.51 ± 0.13 ^{b}	7.35 ± 0.29 ^{c}	**
		Serum ALP(lu/L)			
21	M	127.83 ± 0.34 ^{a}	117.48 ± 0.39 ^{ab}	110.18 ± 1.07 ^{b}	**
	F	125.99 ± 0.27 ^{a}	117.91 ± 0.36 ^{ab}	103.80 ± 0.47 ^{b}	**
	Average	127.34 ± 0.26 ^{a}	113.87 ± 0.26 ^{b}	105.85 ± 0.46 ^{c}	**
35	M	143.57 ± 0.36 ^{a}	121.52 ± 0.35 ^{ab}	100.82 ± 1.80 ^{c}	**
	F	131.87 ± 0.23 ^{a}	116.21 ± 0.23 ^{b}	88.41 ± 0.49 ^{c}	**
	Average	140.67 ± 0.31 ^{a}	118.69 ± 0.21 ^{b}	92.38 ± 0.52 ^{c}	**

Gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** mean a significant difference at p≤0.05 and p≤0.01 levels, respectively.

The AST and ALP in serum of both sexes of 21 days old, high ghrelin gene expressed were significantly ($p<0.01$) higher than those of low gene expressed, whereas ALT serum concentration of both sexes of high gene expressed significantly surpassed low and moderate gene expressed birds (Table 7). At 35 days of age, ALT, AST and ALP concentrations in serum of both sexes of high ghrelin expressed were significantly ($p<0.01$) higher than those of moderate and low ghrelin gene expression. ALT, AST and ALP levels increase with ghrelin gene expression raise at 35 days of age (Table 7). Our results did not match with results of Shahrayar and Lotfi (21) who did not found a significant difference in ALP concentration in serum broiler chickens after *in ovo* injection 50 and 100 ng of ghrelin. The positive correlatation between ghrelin gene expression and ALP concentration is a result of a physiological roles of ghrelin in gastrointestinal tract. which stimulates development and function of gastrointestinal tissue. Several studies in different species observed that ghrelin leads to stomach and intestinal development following ghrelin injection, that leads to increase gastric HCl, which is important to stimulating maturation of enterocytes that plays vital role in activatation ALP (3 , 11 , 18 , 33 , 34).

REFERENCES

1.Al-Hassani, D, H, 2000. Physiology of Poultry. Books house for printing and publishing. Baghdad, Iraq.

2.Al-hassani,D.H. and A. Al-Shukri, 2016. Comparative efficacy of diffent supplements with drinkig water used to alleviate body temperature of heat-stressed broiler chickens. The Iraqi Journal of Agricultural Sciences, 47(Special Issue):12-18·

3.Baintner, K., 2002. Vacuolation in the young. In: Biology of the Intestine in Growing Animals, R. Zabielskil, P.C. Gregory, B. Westrom (ed.). Elsevier. Amsterdam. pp. 55 – 110.

4.Banks, W. A. B. O. Burney,and S. M. Robinson, 2008. Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. Peptides 29: 2061-2065.

5.Banks, W. A. M. Tschop, S. M. Robinson, and M. L. Heiman, 2002. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. Journal of Pharmacology and Experimental Therapeutics.302:822–827.

6.Blevins, J. E. M. W. Schwartz, and D. G. Baskin, 2002. Peptide signals regulating food intake and energy homeostasis. Canadian Journal of Physiology and Pharmacology. 80:396-406.

7.Boswell, T.; and Li, Q. S. Takeuchi, 2002. Neurons expressing neuropeptide Y mRNA in the infundibular hypothalamus of Japanese quail are activated by fasting and co-express agouti-related protein mRNA. Molecular Brain Research 100, 31–42.

8.Chen, L. L. Q. Y. Jiang, X. T. Zhu,; Shu, G. Y. F. Bin, X. Q.Wang; P. Gao, and P.; Y. L. Zhang, 2007. Ghrelin ligand receptor mRNA expression in hypothalamus, proventriculus and liver of chicken (Gallus gallus domesticus): studies on ontogeny and feeding condition. Comparative Biochemistry and Physiology, 147; 893–902.

9.Dezaki, K.; H. Hososda,; M. Kakei, S. Hashiguchi; M. Watanabe; K. Kangawa,; and T. Yada, 2004. Endogenous ghrelin I pancreatic islet restricts insulin release by attenuating Ca2+ signaling in -cells: Implication in the glycemic control in rodents. Diabetes. 53: 3142-3151.

10.Duncan, D. B. 1955. Multiple Range and Multiple F Tests. Biometrics, 11: PP: 1-42.

11.Ghiasi Ghalehkandi, J.; Kumar, M.; Y. Ebrahimnezhad, And R Beheshti, 2012. *In ovo* administration of ghrelin and subsequent intestinal alkaline phosphatase (ALP) activity in broiler chickens. *Advances in Bioresearch*, 3, 9–13.

12. Holmberg; S. K. and S. Mikko; T. Boswell,; R. Zoorob,; and D. Larhammar, 2002. Pharmacological characterization of cloned chicken neuropeptide Y receptors Y1 and Y5. Journal of Neurochemistry. 81:462–471.

13.Ibrahim D.K. and D.H. Al-Hassani. 1997. Effect of acclimation to heat stress during early age and second critical period of adrenal development on broiler performance reared later under high temperature. The Iraqi J. Agri. Sci., 28, 1,: 221- 228.

14.Jensen, J. 2001. Regulatory peptides and control of food intake in non-mammalian

vertebrates. Comparative Biochemistry and Physiology. 128A:471-479

15.Kaiya, H.; S. V. Gayten,; M. Kojima,; H. Hosoda; Y. Kitajima,; M. Matsumoto,; S. Geelissen,; V. M. Darra,; and K. Kangawa, 2002. Chicken ghrelin: purification, cDNA cloning, and biological activity, Endocrinology 143:3445–3463.

16.Kawakami, S. T. Bungo,; R. Ando,; A. Ohgushi; M. Shimojo; Y. Masuda; and M. Furuse, 2000. Central administration of alpha-melanocyte stimulating hormone inhibits fasting- and neuropeptide Y-induced feeding in neonatal chicks. European Journal of Pharmacology. 398:361–364.

17.Kojima, M. H. Hosoda,; Y. Date; M. Nakazato,; H. Matsuo,; and K. Kangawa, 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402 656–660.

18.Korbonits, M. S. A. Bustin,; and M. Kojima, 2001. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. Journal of Clinical Endocrinology and Metabolism. 86:881-887.

19.Kuenzel, W. J. M. M. Beck,; and R. Teruyama, 1999. Neural sites and pathways regulating food intake in birds: A comparative analysis to mammalian systems. Journal of Experimental Zoology. 283:348–364.

20.Livak, K. J. and T. D. Schmittgen, 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method. *Methods* 25(4): 402–408.

21.Lotfi, A.; A. H. Shahryar,; and H. Kaiya, 2013. Effect of in ovo ghrelin administration on hatching results and post-hatching performance of broiler chickens, Livestock Science, 154, 158–164.

22.Maejima, Y.; D. Kohno,; Y. Iwasaki,; and T. Yada, 2011. Insulin suppresses ghrelin-induced calcium signaling in neuropeptide Y neurons of the hypothalamic arcuate nucleus. Aging 3:1092–1097.

23.National Research Council, 1994. National Academy of Science. Nutrient Requirement of Poultry 9th ed. Washington, USA.

24.Phillips-Singh, D.; Q. Li,; S. Takeuchi,; T. Ohkubo,; P. Sharp,; and T. Boswell, 2003. Fasting differentially regulates expression of agouti-related peptide, proopiomelanocortin, preproorexin, and vasoactive intestinal polypeptide mRNAs in the hypothalamus of Japanese quail. Cell and Tissue Research 313, 217–225.

25.Richards, M. P. 2003. Genetic regulation of feed intake and energy balance. Poultry Science. 82:907–916.

26.SAS, 2000. Propriety Software Release 8.1. Statistical Analysis Systems Institute, INC., Cary, North Carolina, USA.

27.Shahryar, A. H.; and A. Lotfi, 2016. Effects of peripheral administration of ghrelin antagonist [D-Lys3]-GHRP-6 on growth performance and blood biochemical indices in broiler chickens. Archives Animal Breeding., 59, 113–119.

28.Shintani, M.; Y. Ogawa,; K. Ebihara,; M. Aizawa-Abe,; F. Miyanaga,; K. Takaya,; T. Hayashi,; G. Inoue,; K. Hosoda,; M. Kojima; K. Kangawa,; and K. Nakao, 2001. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50: 227–232.

29.Tachibana, T.; K. Sugahara,; A. Ohgushi,; R. Ando,; S. Kawakami,; T. Yoshimatsu,; and M. Furuse, 2001. Intracerebroventricular injection of agouti-related protein attenuates the anorexigenic effect of alpha-melanocyte stimulating hormonein neonatal chicks. Neuroscience Letters. 305:131–134.

30.Takeuchi, S.; K. Teshigawara,; and S. Takahashi, 2000. Widespread expression of agouti-related protein (AGRP) in the chicken: A possible involvement of AGRP in regulating peripheral melanocortin systems in the chicken. Biochimica et Biophysica Acta 1496:261–269.

31.Woods, S. C.; R. J. Seeley,; Porte, Jr. D.; and M. W. Schwartz, 1998. Signals that regulate food intake and energy homeostasis. Science 280:1378–1383.

32.Yada, T.; K. Dezaki,; H. Sone,; M. Koizumi,; B. Damdindoj,; M. Nakata,; and M. Kakei, 2008. Ghrelin regulates insulin release and glycemia: Physiological role and therapeutic potential. Current Diabetes Reviews. 4: 18-23.

33.Yakabi, K.; J. Kawashima,; and S. Kato, 2008. Ghrelin and gastric acid secretion.

World Journal of Gastroenterology 14(41): 6334-6338.

34.Yoshimura, Y.; C. Tsuyuki,; K. Subedi,; H. kaiya,; T. Sugino,; and I. Naoki, 2009. Identification of ghrelin in fertilized eggs of chicken. Journal of Poultry Science, *46:* 257-259.